G—103

TEMPERATURE RELATIONS OF APPLE-ROT FUNGI

BY

CHARLES BROOKS AND J. S. COOLEY

BUREAU OF MYCOLOGY
15 JUL 1921

Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. VIII, No. 4 : : : : Washington, D. C., January 22, 1917

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1917

TEMPERATURE RELATIONS OF APPLE-ROT FUNGI[1]

By CHARLES BROOKS, *Pathologist*, and J. S. COOLEY, *Assistant Pathologist, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The retarding effect of low temperatures on plant activities is a matter of general knowledge, and the principle has had a very general application in the storage of fruits. The better preservation of the fruit at low temperatures is due both to the slowing up of the activities of the fruit itself and to the checking of fungus and bacterial growth. This paper deals particularly with the latter phase of the storage problem. An effort has been made to carry on the investigations in a manner that would make the data of general physiological interest as well as of practical value in fruit storage.

HISTORICAL REVIEW

Eustace[2] made one of the first studies of the storage-rot problem in this country. Several varieties of apples (*Malus sylvestris*) were inoculated with *Alternaria* sp., *Glomerella rufomaculans* (Berk.) Sp. and Von Schr., *Sphaeropsis malorum* Pk., *Penicillium glaucum* Lk., *Sclerotinia fructigena* (Pers.) Schrt., and *Cephalothecium roseum* Cda.; and similar inoculations were made on agar petri plates. After two months at a temperature that varied from 30° to 33° F. and averaged 32° *Penicillium glaucum* had made a good growth on both the agar and the apples and *Alternaria* sp. had made a slight growth on the apples. None of the other fungi had produced any growth, but all of them developed rapidly when removed to a temperature of 70°. In another experiment inoculated fruit and agar plates were placed in a storage room in which the temperature ranged from 35° to 56° and averaged 47°. All of the fungi made a good growth. At a temperature of 48° to 69° the development of the fungi was still more rapid.

Schneider-Orelli[3] made experiments with *Gloeosporium herbarum* Lk., *Mucor piriformis* Fisch., *Penicillium glaucum*, *Botrytis cinerea* Pers., *Monilia fructigena* Pers., *Fusarium putrefaciens* Osterw., *Gloeosporium album* Osterw., *Gloeosporium fructigenum* Berk., and *Rhizopus nigricans*

[1] Studies on Fruit Rots and Spots.—I.

[2] Eustace, H. J. Investigations on some fruit diseases. N. Y. State Agr. Exp. Sta. Bul. 297, p. 31-48, 7 pl. 1908.

[3] Schneider-Orelli, Otto. Versuche über die Wachstumsbedingungen und Verbreitung der Fäulnispilze des Lagerobstes. *In* Landw. Jahrb. Schweiz, Jahrg. 25, Heft 3, p. 225-246. 1911. *Also in* Centbl. Bakt. [etc.], Abt. 2, Bd. 32, No. 6/12, p. 161-169. 1912.

——— Zur Kenntnis des mitteleuropäischen und des nordamerikanischen Gloeosporium fructigenum. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 32, No. 13/19, p. 459-467. 1912.

Journal of Agricultural Research, Washington, D. C.
gx

Vol. VIII, No. 4
Jan. 22, 1917
Key No. G—103

Ehrbg. He grew the fungi on gelatin in petri plates at temperatures of 0°, 4.5°, 9.5°, 14°, and 18° C. At the end of 35 days all but the last two of these fungi had made a measurable growth at 0°, *Botrytis cinerea* making the most vigorous growth. At 4.5° *Rhizopus nigricans* and *Gloeosporium fructigenum* had produced small colonies at the end of 35 days.

Penicillium glaucum, *Botrytis cinerea*, *Monilia fructigena*, and *Gloeosporium album* were inoculated into apples and the fruit stored in one case at 4.5° and in another at 14° C. The diameter of the rots produced at the end of two and three weeks is given. The first three fungi made a fair growth at the lower temperature.

In a later paper Schneider-Orelli [1] reported temperature studies on different species or strains of *Gloeosporium fructigenum*. He found that the European form had lower optimum, maximum, and minimum temperatures than the American form. At 5° C. the European form, when grown in gelatin in petri plates, produced a colony 0.4 cm. in diameter in 12 days and a colony 3.7 cm. in diameter in 35 days, while the American form had made no growth at the end of 35 days.

Edgerton [2] made a study of the behavior of various American species and strains of Glomerella. He found that there were two different strains of Glomerella on the apple, a rapid-growing southern form and a slow-growing northern form. The former had an optimum temperature of 27° to 29° C. and a maximum temperature above 37.5°, produced scanty aerial mycelium in culture, formed cankers on apple limbs, besides rotting the fruit, and very commonly produced perithecia on the host and in culture. The latter had an optimum temperature of 24° to 25° and a maximum temperature of 34° to 35°, produced a greater abundance of white aerial mycelium in culture, and apparently did not produce cankers on apple limbs, though it rotted the fruit. He thought the former should be called "*Glomerella cingulata*" and that the latter should retain the name "*Gloeosporium fructigenum*" given by Berkeley. He considered the latter fungus probably identical with the European one.

Ames [3] determined the germination and growth of *Monilia fructigena*, *Penicillium digitatum* (Fr.) Sace., *Rhizopus nigricans* Ehrenb., *Glomerella rufomaculans*, and *Cephalothecium roseum* at various temperatures. The germination tests were made by means of Van Tieghem cell drop cultures. At 1° C. only the first two of these fungi germinated within 245 days, while at 3° to 4° all but *Cephalothecium roseum* germinated within 200 days. The growth of the fungi was tested on bean agar, and in this series *Penicillium glaucum* was substituted for *P. digitatum*. None of the fungi except *P. glaucum* made any growth at a temperature of 1° to 2° and

[1] Schneider-Orelli, Otto. Op. cit., 1912.

[2] Edgerton, C. W. The physiology and development of some anthracnoses. *In* Bot. Gaz., v. 45, no. 6, p. 393, 402–403. 1908.

——— Effect of temperature on Glomerella. *In* Phytopathology, v. 5, no. 5, p. 247–259, 4 fig. 1915.

[3] Ames, Adeline. The temperature relations of some fungi causing storage rots. *In* Phytopathology, v. 5, no. 1, p. 11–19. 1915.

this produced but very small colonies. At a temperature of 3° to 4° *Monilia fructigena* made a scant development, and at 9° to 10° all of the fungi made some growth, the species of Monilia and Penicillium both fruiting within 11 days.

EXPERIMENTAL INVESTIGATIONS

APPARATUS

The experiments reported in this paper were carried out in refrigerator boxes constructed especially for the work. The low temperatures were obtained by means of a sulphur-dioxid refrigerating machine. This machine was stopped and started automatically by means of a tumbler switch and a 3-step starter and the brine thus kept within a range of 2° C. In the cooler months it was kept at a temperature of −8° to −6°, but in the warmer months it was necessary to carry it one or two degrees colder in order to obtain the required refrigeration. In testing out regulators it was found that a short bimetallic form was entirely unsatisfactory in humid weather, as the moisture condensed on the cold metal at the contact points and caused considerable trouble in the operation of the machine. Later, a regulator was used in which the points of contact were placed a long distance from the brine and the make-and-break brought about by means of a column of mercury under pressure; this was found entirely satisfactory. The brine was circulated through the pipes in the boxes by means of a rotary pump which was kept in operation all the time. Different amounts of brine were thrown into the different boxes by means of control valves on the pipes at the entrance to the boxes, thus making it possible to maintain a different temperature in each box.

The walls of the boxes were 5¾ inches thick, sheet cork and heavy paper being used as insulating material. A galvanized-iron box was placed inside the wooden box. On the sides and bottom there was a 3-inch air space between this inner metal box and the outer box, while at the top there was a space of less than an inch between the metal box and the brine pipes. The inner box was 2 feet high and approximately 3 feet square. The air was circulated in each double box by means of blower fans that were operated constantly, one fanning the air over the brine pipes and through the air space between the two boxes, and the other stirring the air of the inside box. The air from this latter fan was carried across the top of the box by means of a special duct and discharged on the opposite side, thus insuring a complete circulation. A closely fitting slide door in the metal box made it possible to circulate the air from over the brine pipes directly through the inner box if quick cooling was desired. The temperature of the air space between the boxes was always kept a few degrees lower than that desired in the inner box, and the latter brought to the required temperature by the use of electric lamps. Since the brine was kept at a practically constant tem-

perature and was distributed to the different boxes according to the cooling required, only a small amount of heating was necessary to maintain the desired temperature in the inner boxes, two 60-watt electric lamps being sufficient in any one box. These lamps were thrown on and off by means of ordinary telegraphic relays operated by mercury regulators. The lamps were placed in the duct that carried the air across the inner box, thus preventing local heating and also the access of light to the fruit.

The apparatus as described gave very satisfactory results. The circulation of the air was sufficient to maintain uniform temperatures throughout the box and the regulation sufficiently delicate to secure practically straight lines on the thermograph charts. The use of the inner box and the double circulation of air made it possible to maintain higher humidities than could have been done if the air that circulated over the fruit had been exposed to the drying effects of the brine pipes.

In the 5° compartment no inner box was used; and although the air was stirred with a blower fan, the top of the box was about one degree warmer than the bottom. The temperature was regulated as with the other boxes and the lamps were inclosed so as to prevent lighting the compartment.

For obtaining the 30° temperature mentioned in the experiments, an ordinary water-jacket incubator with electric heater was used. The air was not stirred and was about a half degree too warm in the top of the chamber and a half degree too cold in the bottom.

FUNGI

The following fungi were used in the experiments: *Alternaria* sp., *Botrytis cinerea*, *Cephalothecium roseum*, *Fusarium radicicola* Wollenw., *Glomerella cingulata* (Atk.) S. and S., *Neofabraea malicorticis* (Cord.) Jackson, *Penicillium expansum* (Lk.) Thom, *Pestalozzia funerea* Desm., *Sclerotinia cinerea* (Bon) Schroeter, *Sphaeropsis malorum*, *Trichoderma* sp., and *Volutella fructi* S. and H.

They were obtained from decayed fruit collected in various parts of the United States, and were selected as the most virulent organisms from a much larger number that were found capable of producing more or less decay on ripe pome fruits. Each fungus was tried on various culture media to find a substance upon which it would make a good growth and good spore production. Most of the fungi were kept in culture on corn-meal agar. *Fusarium radicicola* was usually grown on potato agar and *Penicillium expansum* on either apple or potato agar. Only cultures that appeared to be in a vigorous condition were used. The inoculations were in most cases made with spores, but with fungi such as *Sphaeropsis malorum* that did not fruit readily in culture, mycelium was sometimes used. So far as observed, no contrasts were seen between the results with spore inoculations and those with mycelium.

INOCULATIONS ON FRUIT

Great care was taken in selecting the fruit for inoculation, as it was found by preliminary experiments that the rapidity of the rot depended greatly upon the variety and maturity of the apples. Only fresh, crisp fruit was used, and the apples for a particular experiment were all of the same lot. Uniformity was obtained by selecting seven apples (or as many as there were temperatures) that were similar in size and degree of maturity and distributing these one each in the seven moist chambers that were to be placed at the different temperatures, and repeating the process until the desired number of apples was obtained. Except where otherwise stated, four apples were used at each temperature in each set of experiments.

Care was taken to have the fruit in as sterile a condition as possible. The fleshy part of the stem next to the absciss-layer was found especially subject to infection by various fungi, and in order to eliminate the possibility of contamination from this source the apple stems were always clipped off close to the flesh of the fruit as the first step in preparing for inoculation. The fruit was then washed in soap and water, and this was followed by immersion in mercuric chlorid (1: 1,000) for three minutes, and, finally, the fruit was given a thorough rinsing in sterile water. Inoculations were made by forcing the spores or mycelium down into the flesh of the fruit by means of a platinum needle. The fruit was stored in sterile moist chambers and sufficient moist filter paper added to keep the air in a practically saturated condition. The fruit was cooled to the desired temperatures within a few hours after storage, even in the coldest chambers. Notes were usually taken on the fruit at intervals of one week. The records were made as rapidly as possible, so that the fruit was kept out of the particular box only for a few minutes. At the lower temperatures the notes could be taken still more rapidly in the early stages of the experiment, as the results were usually entirely negative.

The culture of *Sclerotinia cinerea* used in the first set of experiments (see fig. 1) was from a peach plum from Wenatchee, Washington; one of those used in the second experiments (see fig. 2 and 3) was from a prune from Vancouver, Washington, and the other from an apple from West Virginia. The characteristics of the different cultures indicated that *S. cinerea* had been isolated in each case, but the fungus from apples proved a more vigorous rot organism than the others.

With most of the fungi the various inoculations started off together; but with those that were scarcely able to attack the apple, such as the species of Alternaria, Cephalothecium, Fusarium, Pestalozzia, and Trichoderma, there was sometimes considerable difference in the time of starting of different inoculations under the same conditions, especially as the minimum temperature for the particular fungus was approached. In such cases all of the inoculations that were counted at the last of the

experiment in obtaining the average rot were also counted at the first, even though the record was zero.

The comparative behavior of the different fungi is shown in figures 1, 2, and 3. The base line shows the temperature in degrees centigrade; [1] the perpendicular the diameter of the rots in millimeters. The curves are based on the average weekly increase in the diameter. The results given in figure 1 were obtained from inoculations made on March 27, 1915; those of figures 2 and 3 from inoculations made on January 21, 1916.

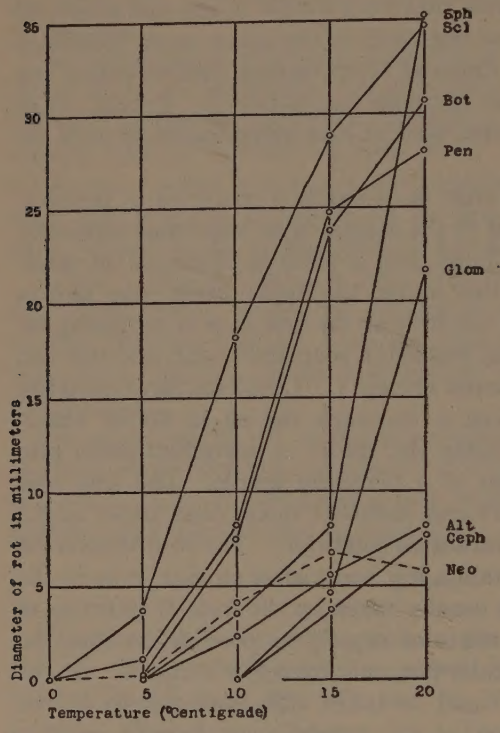

FIG. 1.—Graph showing the development of rot on Ben Davis and York Imperial apples. The curves show the average weekly increase in diameter and are based on records taken two weeks after inoculation.[2]

In the first series of experiments the moist chambers were placed at once at the temperatures desired; in the second they were allowed to remain at 20° for 24 hours before removal to the storage temperatures. A study of the curves will show that some of the rots started off more rapidly at 0° and 5° in the second series of experiments than in the first, but different varieties were used in the two sets of experiments, making it impossible to attribute the contrast entirely to difference in time of storage. The effect of delayed storage will be discussed later in the paper.

It may be noted that some of the curves in figures 2 and 3 are relatively high at 5°. As has already been mentioned, the top of the 5° chamber was 1° warmer than the bottom. During the last experiment this box was used for the storage of other fruit, sometimes resulting in the placing of some of the experimental fruit in close proximity to warm

[1] Temperature equivalents:

°C.	°F.	°C.	°F.	°C.	°F.
30	86	15	59	5	41
25	77	10	50	0	32
20	68				

[2] Abbreviations used in text figures; Alt.=*Alternaria* sp.; Bot.=*Botrytis cinerea;* Ceph.=*Cephalothecium roseum;* Fus.=*Fusarium radicicola;* Glo.=*Glomerella cingulata;* Neo.=*Neofabraea malicorticus;* Pen.=*Penicillium expansum;* Pes.=*Pestalozzia funerea;* Pho.=*Phoma pomi;* Scl.=*Sclerotinia cinerea;* Sph.=*Sphaeropsis malorum;* Tri.=*Trichoderma* sp.: Vol.=*Volutella fructi.*

packages and in the crowding of some of it nearer to the top of the chamber. While the thermograph records did not indicate any error, it is probable that in the last experiment some of the fruit in the 5° chamber was exposed to a temperature about 1° warmer than indicated.

The behavior of the more virulent rot fungi has been practically the same on one variety of apples as on another, but with the weaker organisms the variety of apples has had an important modifying effect upon the temperature results. *Cephalothecium roseum* produced a small amount of rot on the Yellow Newtown at 5°, but was unable to attack the Winesap, York Imperial, and Ben Davis at this temperature or to produce any significant growth on these varieties at 10°. *Fusarium radicicola* was able to rot Newtowns at a lower temperature than Winesap. *Trichoderma* sp. rotted the York Imperial at 20° but did not rot the Ben Davis.

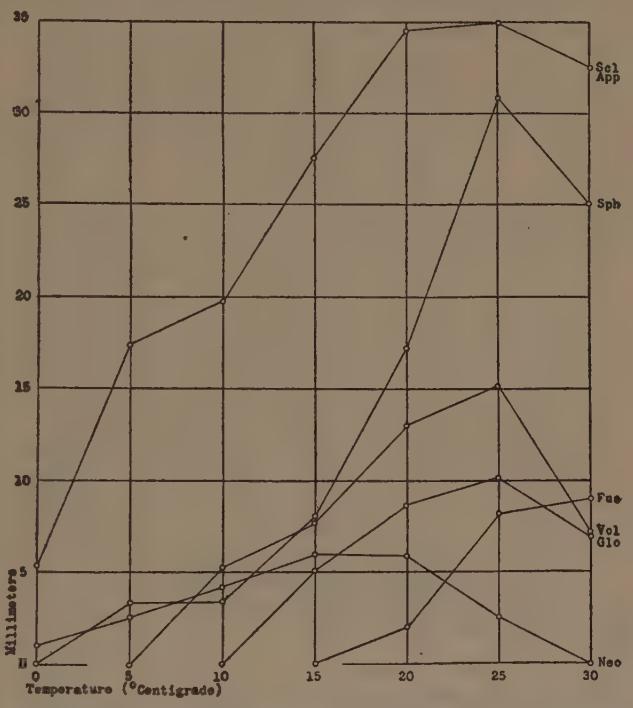

FIG. 2.—Graph showing the development of rot on Yellow Newtown apples. The curves show the average weekly increase in diameter. With *Sclerotinia cinerea*, *Sphaeropsis malorum*, and *Volutella fructi* they are based on records made two weeks after inoculation; with the other fungi on records made three weeks after inoculation.

The apparent difference in susceptibility of varieties may have been partly due to differences in ripeness of the fruit, as there seemed to be no satisfactory way of determining the comparative maturity of the different varieties. It was repeatedly observed that a slight increase in the ripeness of apples of a particular variety caused a definite increase in susceptibility to rot. This modifying effect of maturity of the fruit was particularly noticeable as the minimum temperature for the fungus was approached. In this connection it may be mentioned that in cases where fungi had apparently lost some of their rot-producing power as a result of long culturing in the laboratory this attenuation was particularly evident at the lower temperatures.

The contrasts between the temperature responses of the different fungi are very striking. *Sclerotinia cinerea* is the most rapid rot-producing organism tested and is less inhibited by cold than most of the other fungi. *Sphaeropsis malorum* is in general more rapid than *Penicillium expansum;* but the former is practically inhibited at 0°, while the latter can make little growth at 30°. The low optimum and slow rate of growth with *Neofabraea malicorticis* are particularly interesting.

The contrasting results at the different temperatures are shown in graphic manner in Plates 2 and 3.

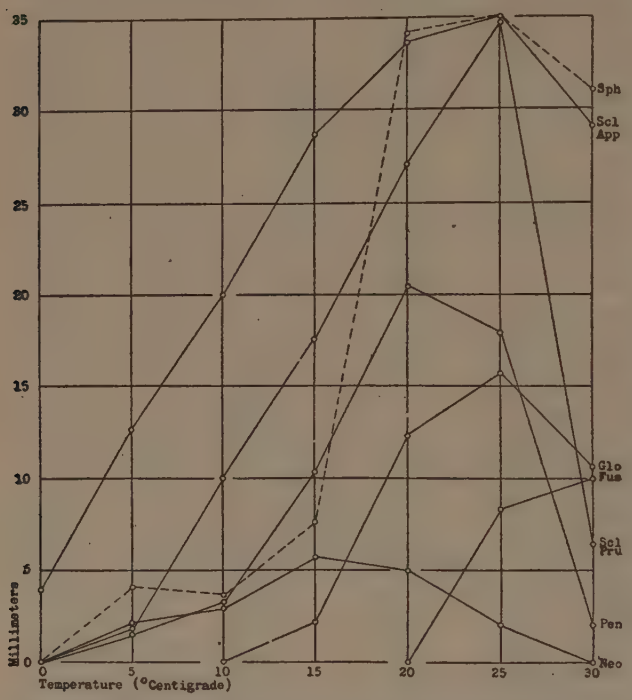

FIG. 3.—Graph showing the development of rot on Winesap apples. The curves show the average weekly increase in diameter. With *Sclerotinia cinerea*, *Sphaeropsis malorum*, and *Volutella fructi* they are based on records made two weeks after inoculation; with the other fungi on records made three weeks after inoculation.

In figures 1, 2, and 3 the diameter of the rot is taken as the basis of comparison. This does not give the real contrast in size of rot or in the amount of work done by the fungus. A comparison of the temperature effects on a chemical or physiological basis would require that the volume of the rot be considered rather than the diameter. Similar volumes have the same ratio to each other as the cubes of their like dimensions, but unfortunately the masses of rotted tissue do not have similar shapes at the different stages in their development. The fungus can not spread outward, and it usually spreads laterally more rapidly than it does toward the core, thus making the area of the rot a fairly close index of its volume. In figure 4 the curves are plotted on the basis of the average rot areas at the different temperatures. It will be noted that in most cases the increase in growth with a 10° rise in temperature comes within the range of the Van't Hoff law; but there are several striking exceptions, *Sphaeropsis malorum* producing a rot area 24 times

as great at 25° as at 15° and *Penicillium expansum* showing a similar contrast between 10° and 20°. It was also found that Botrytis produced a rot area 25 times as great at 20° as at 10° on the Ben Davis and 57 times as great on the York Imperial.

The progressive development of the rots is shown in figures 5 to 12. The curves are based on the diameter of the rot at the various temperatures after the number of weeks indicated. The temperatures are given on the base line and the diameter of the rots in millimeters on the perpendiculars.

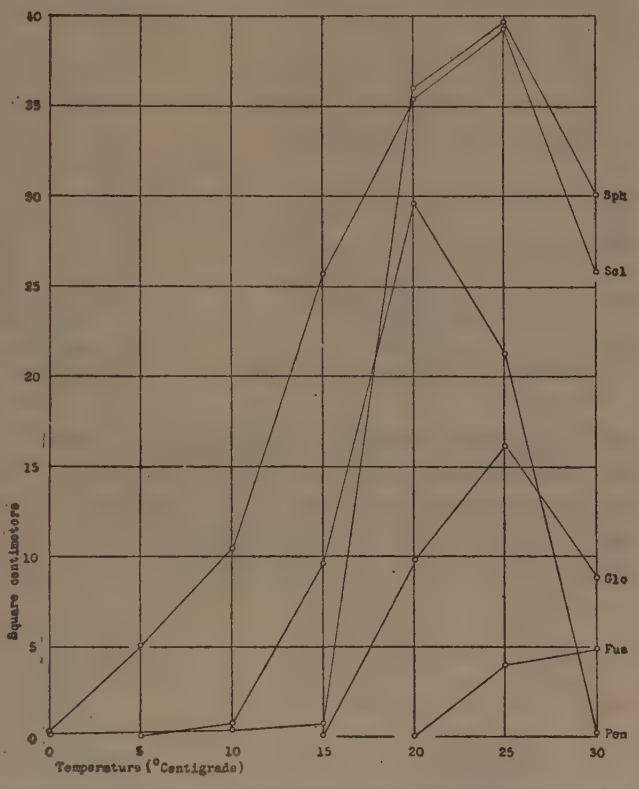

FIG. 4.—Graph showing the development of rot on Winesap apples based on the area instead of the diameter of the rot.

It will be observed that in general the rate of rotting increases with time. In some cases there appear to be exceptions to this rule, but it should be remembered that the curves are plotted on the basis of diameter and not volume, thus giving a decreased value to the growth rate in the larger rots. It is of particular interest to note that the conditions for rapid growth are acquired much more quickly at higher temperatures than at the lower ones. At 20° and 25° most of the fungi became active rot-producing agencies within a week, but as the temperatures became lower, longer and longer periods of incubation were required. When the fungi had once become well established at the lower temperatures, however, the rapidity of the rotting was often most surprising. Good examples of this necessity of incubation may be seen by contrasting the growth of *Sphaeropsis malorum* at 15° and 20° during the first week with that of the second, the growth of *Sclerotinia cinerea* at 0° and 5° during the same two weeks, or the growth of *Penicillium expansum* at 0° and 5° in the first five weeks as compared with that of the last three.

The minimum temperature for some of the fungi seems to be practically absolute, but for others it varies with the length of time the experiment has run. *Fusarium radicicola* was unable to develop at 15° and *Glomerella cingulata* at 10° even after long periods of storage. *Neofabraea malicorticis* and *Penicillium expansum* had produced no rot at 10°

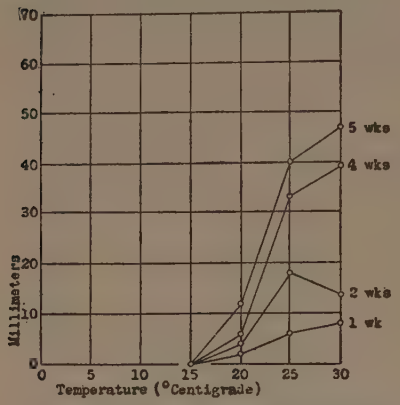

FIG. 5.—Graph showing the development of the rot caused by *Fusarium radicicola* on Yellow Newtown apples.

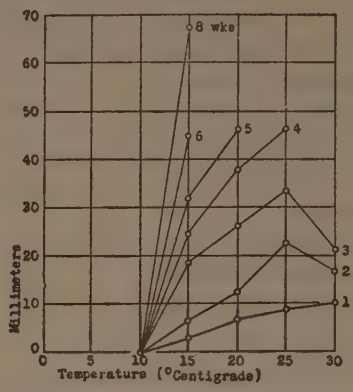

FIG. 6.—Graph showing the development of the rot caused by *Glomerella cingulata* on Yellow Newtown apples.

at the end of two weeks nor *Sphaeropsis malorium* at 15° at the end of one week, but later all made a fair growth at 0°. At the end of two weeks *Alternaria sp.*, *Botrytis cinerea*, and *Volutella fructi* had made no evident growth at 5°, but later developed at 0°. *Sclerotinia cinerea* was the most active at low temperatures of any of the fungi tested, making

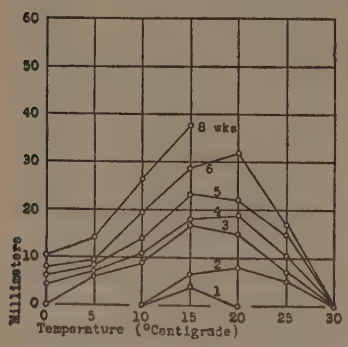

FIG. 7.—Graph showing the development of the rot caused by *Neofabraea malicorticis* on Winesap apples.

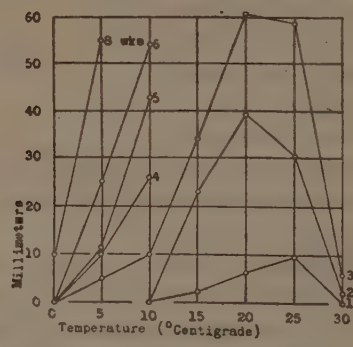

FIG. 8.—Graph showing the development of the rot caused by *Penicillium expansum* on Winesap apples.

a fair growth at 0° by the end of the second week. Further data on the behavior of the various fungi at 0° will be given under the heading of commercial cold storage.

Fusarium radicicola had an optimum at 30° or above; *Glomerella cingulata*, *Sphaeropsis malorum*, *Sclerotinia cinerea*, and *Volutella fructi*

at 25°; *Penicillium expansum* at 20°; and *Neofabraea malicorticis* at 15°. It is interesting to note that the highest summer temperatures are unfavorable to most of the vigorous rot organisms. The fact appears to explain numerous failures that the writers have had with fruit inoculations made in the hottest weeks of the summer. The graphs show that high

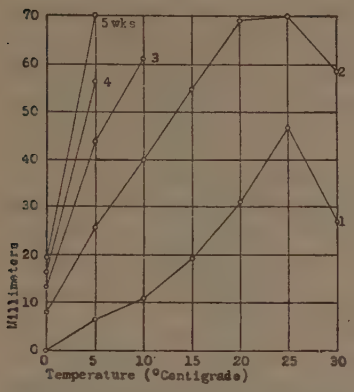

FIG. 9.—Graph showing the development of the rot caused by *Sclerotinia cinerea* (apple) on Winesap apples.

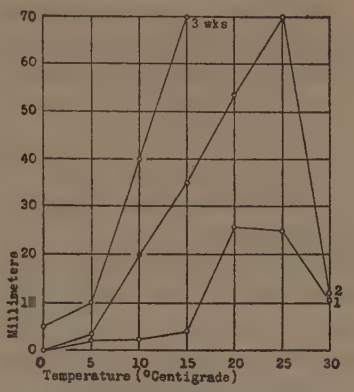

FIG. 10.—Graph showing the development of the rot caused by *Sclerotinia cinerea* (prune) on Winesap apples.

temperatures had a greater retarding effect upon *Neofabraea malicorticis* than upon any of the other fungi. A further illustration of this was found in the removal of inoculated fruit from low temperatures to higher ones. When apples on which *Sphaeropsis malorum*, *Penicillium expansum*,

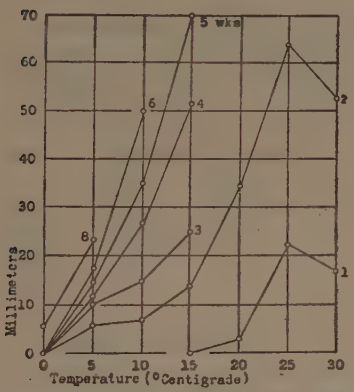

FIG. 11.—Graph showing the development of the rot caused by *Sphaeropsis malorum* on Yellow Newtown apples.

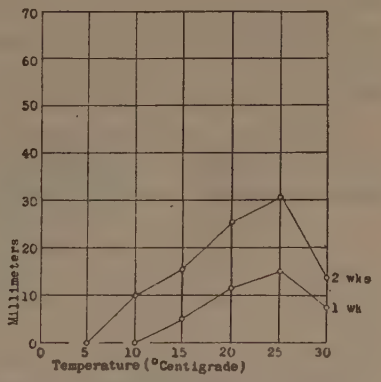

FIG. 12.—Graph showing the development of the rot caused by *Volutella fructi* on Yellow Newtown apples.

and *Neofabraea malicorticis* had produced small rot areas at 0° were removed to laboratory temperature (about 25°), the first two fungi rotted the fruit all the more rapidly at the higher temperature, but the growth of the last named was apparently entirely checked.

The temperatures used were not high enough to determine the maximum for most of the fungi. *Neofabraea malicorticis* refused to grow at 30° and *Penicillium expansum* made but a scant growth at that temperature.

ORCHARD-INFECTED FRUIT

Experiments were also made with *Sphaeropsis malorum* and *Glomerella cingulata*, using rots that were produced by natural infection instead of by artificial inoculation. The condition of the apples at the beginning of the experiment is shown in Plate 1. The rots at this time were mere specks, the largest being only a few millimeters in diameter, and most of them much smaller. The records are based on the increase in the diameter of the rot, the original diameter being subtracted where the initial rot was of measurable size. The blackrot experiments were made on Grimes Golden and Northwestern Greening apples. The results were so uniform with the two varieties that one summary is given for both. The bitter-rot experiments were on Ben Davis apples from Virginia. Part of the apples were placed in large moist chambers, the others in wire baskets. About a peck of apples was used at each temperature for the open package and about half as many in the closed moist chambers. The air in the moist chambers was kept practically saturated. The apples in the open packages were exposed in air that ranged from 50 to 70 per cent relative humidity. The results are given in Tables I and II.

TABLE I.—*Results of natural infection with Sphaeropsis malorum—Experiment started on September 13, 1915*

Temperature.	Container.	Diameter of rot (in millimeters) after—		
		10 days.	24 days.	38 days.
C				
20 to 26, averaging 21	Open basket	23.7	23.7	.....
	Closed chamber	27.2	41.5	.....
15	Open basket	6.6	24.1	.....
	Closed chamber	7.8	27.5	30.0
10	Open basket	1.1	5.5	14.4
0	Open basket	4.2	7.8	13.1
	Closed chamber	0	8	10.5

TABLE II.—*Results of natural infection with Glomerella cingulata*

Temperature.	Container.	Experiment started on September 8. Diameter of rot in millimeters after—				Experiment started on September 28. Diameter of rot in millimeters after—			
		15 days.	29 days.	36 days.	43 days.	9 days.	16 days.	23 days.	37 days.
°C.									
39[a]	Closed chamber	.....	.....	.....	.....	4.4	.....	.....	.....
35[a]	do	.....	.....	.....	.....	9.9	15.5	.....	.....
28[a]	do	.....	.....	.....	.....	6.0	16.3	.....	.....
20 to 26, averaging 22.	Open basket	28.7	.....	.....	.....	13.6	24.3	.....	.....
	Closed chamber	.....	.....	.....	.....	7.1	25.2	.....	.....
15	Open basket	3.6	9.7	18.0	28.0	.....	.....	.....	.....
	Closed chamber	0	20.5	31.5	42.0	3.7	7.8	.....	.....
10	Open basket	0	2.2	.....	6.2	.....	.....	.....	.....
	Closed chamber	0	4.2	.....	8.7	0	0	0.9	4.7
5	Open basket	0	0	.....	0	.....	.....	.....	.....
	Closed chamber	0	0	.....	0	0	0	0	0
0	Open basket	0	0	.....	0	.....	.....	.....	.....
	Closed chamber	0	0	.....	0	0	0	0	0

[a] The three higher temperatures were not constant. At these temperatures the apples soon broke down internally, making it difficult to obtain reliable data.

In the later stages of the experiment the development of the rots appeared to be more rapid in the moist chambers than in the open packages. This was probably due to the fact that the exposed apples became considerably withered as the experiment proceeded and were probably not so easily broken down by the rot fungi.

The results with *Sphaeropsis malorum* are in close agreement with those obtained in the artificial inoculation experiments. This was also true of *Glomerella cingulata*, with the exception that there was no growth at 10° with the artificial inoculations while a slight growth was obtained at that temperature with natural infections. The development at this temperature, however, occurred only with rots that were several millimeters in diameter at the beginning of the experiment.

After two months' exposure to temperatures of 0°, 5°, or 10° infections of *G. cingulata* developed rapidly when the fruit was removed to a temperature of 25°.

APPLE ROTS IN COMMERCIAL COLD STORAGE

Experiments were also made with apples in a commercial cold-storage plant, in which the temperature was held at 0° C. (32° F.). The apples (from the Shenandoah Valley of Virginia) were gathered on October 6, were received in Washington on October 16, and were inoculated on October 19. They were not washed, sterilized, or treated in any way before inoculation. The stems were not recmoved, as was the case in other inoculation experiments. Fruit was selected that was of uniform size and quality and apparently in perfect condition. Ten York Imperial and ten Arkansas apples were inoculated with each fungus by

means of needle punctures. A like number of each variety of apples were allowed to stand for a few minutes in water that had been abundantly inoculated with spores or mycelium from cultures of the particular fungus. The fruit was thus well covered with possible sources of infection. The treated apples were divided into two lots, placed in paper bags, and packed in the middle of barrels with other apples around them. Half of the apples were taken immediately to cold storage, while the other half were kept in the laboratory for one week and then taken to cold storage. They were removed from storage on February 28, and notes were taken immediately. The results are given in Table III.

TABLE III.—*Results of the development of rot on Arkansas and York Imperial apples in commercial cold storage. Inoculations made on October 19; notes taken on February 28*

Fungus.	Average diameter of rot (in millimeters).							
	Inoculated by puncture.				Washed in spore suspensions.			
	Immediate storage.		Storage delayed 7 days.		Immediate storage.		Storage delayed 7 days.	
	Arkansas.	York Imperial.	Arkansas.	York Imperial.	Arkansas.	York Imperial.	Arkansas.	York Imperial.
Alternaria sp. 399	0	0	6	6	0	0	0	0
Alternaria sp. 365	0	0	0	0	0	0	0	0
Aspergillus niger	0	0	23	20	0	0	0	0
Botrytis cinerea	0	0	0	0	0	0	0	0
Cephalothecium roseum	6	7	8	8	0	0	0	0
Fusarium radicicola	0	0	7	35	0	0	0	0
Glomerella cingulata	0	0	9	21	0	0	0	0
Mucor stolonifer	0	0	0	0	0	0	0	(a)
Neofabraea malicorticis	12	15	12	18	0	0	0	0
Penicillium expansum	0	0	3	57	0	0	(b)	0
Sphaeropsis malorum	5	16	25	26	0	0	0	0
Sclerotinia cinerea	75	72	75	75	0	0	0	0
Volutella fructi	0	0	6	7	0	0	0	0

[a] One 45, others 0. [b] One 75, others 0.

The contrasts between immediate and delayed storage are quite striking. The species of Aspergillus, Fusarium, Glomerella, Volutella, Alternaria, and Penicillium produced no rot when the fruit was immediately stored at 0°; while, when storage was delayed for seven days, all produced more or less decay. With the first three of these probably all or nearly all of the rot was produced before the fruit was cooled. This may have been true also of *Alternaria* sp. and *Volutella fructi*, but could not have been the case with *Penicillium expansum* on York Imperial apples, as the experiments already reported show that it is not able to produce rots 57 mm. in diameter in one week. The fact that such an important storage-rot as *P. expansum* failed to start when placed at 0°

but continued to grow when given a start at a higher temperature emphasizes the importance of immediate storage. It is interesting to note how little effect the delay in storage had upon *Cephalothecium roseum* and *Neofabraea malicorticis*. This is in agreement with the temperature responses shown in figures 2 and 7. The growth of *Sphaeropsis malorum* was delayed by immediate storage, and this was probably true of *Sclerotinia cinerea* also; but with the latter fungus no contrast was evident at the end of the experiment, as the fruit was practically completely rotten in the immediate as well as in the delayed storage.

It should be noted that the species of Alternaria, Botrytis, Penicillium, and Volutella used produced no rot when stored immediately at commercial cold-storage temperature, while all have caused more or less rot at 0° in laboratory experiments, *Botrytis cinerea* and *Penicillium expansum* producing rots 1 or 2 cm. in diameter in less than five weeks. It is possible that the air in the cold-storage plant sometimes dropped slightly below 0° C. and that the explanation of the above contrast is to be found in differences in temperature, but the writers are of the opinion that it was due to differences in the maturity of the fruit; as the apples used in the commercial cold-storage experiments were much greener than any of those used in the laboratory tests. The importance of maturity of fruit in determining the amount of rot at low temperatures and the minimum temperature for the fungus has already been pointed out.

It is interesting to note the almost entire absence of rot in the case of the apples washed in spore suspensions, only two apples out of the total of 140 becoming infected at all and these two specimens occurring in the case of delayed storage. The results strongly emphasize the importance of avoiding punctures and other injuries on fruit that is to be held in storage.

TEMPERATURE EXPERIMENTS ON CULTURE MEDIA

The various rot fungi were also tested at different temperatures on several different culture media. The behavior of the fungi on the fruit is of special interest because it is on this medium that they become of economic importance; but the apple is a living organism and when fruit is kept in storage for any length of time, its condition at a high temperature will become very different from that of similar fruit kept at low temperatures. Consequently, in determining the behavior of fungi at different temperatures one must consider two living organisms, the parasite and the host, the former usually being favored by high temperatures and the latter being continually weakened by them. Culture media probably remains practically constant at the different temperatures used and therefore furnishes a fairer test of the temperature responses of the different fungi. It has been difficult, however, to get one medium which was satisfactory for the growth of all the fungi.

FLASK EXPERIMENTS.—In one experiment the fungi were grown on liquid media in 100 c. c. Erlenmeyer flasks. About 50 c. c. of liquid were poured into each flask. Dox's solution plus 2 per cent of dextrose was used with all the fungi except the species of Glomerella and Neofabraea. These did not grow well on this solution, so apple juice about +5 was used as a substitute. The flasks were placed at the particular temperatures immediately after inoculation. The experiment was started on April 16 and was concluded two weeks later. The fungus growth was filtered out by means of filter papers that had previously been dried and accurately weighed. After the fungus mass had been dried to constant weight, a second weighing was taken, the difference between the two weighings representing the dry weight of the fungus. The results are shown in figure 13.

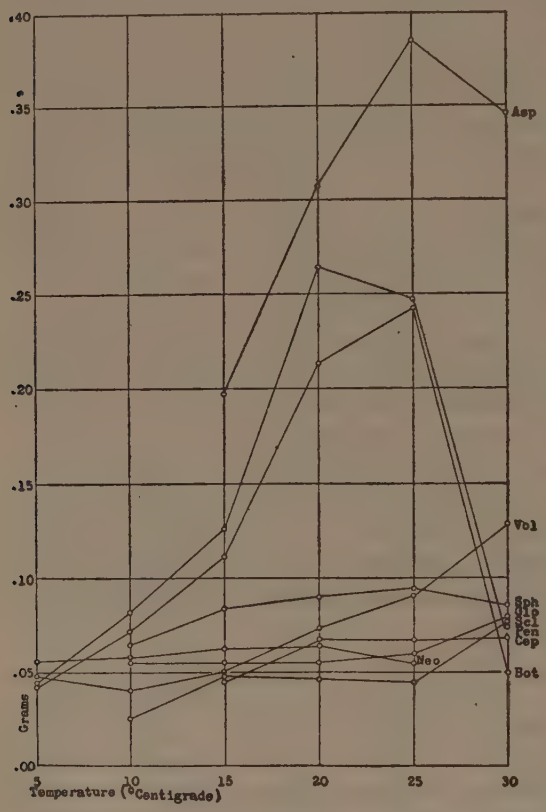

FIG. 13.—Graph showing the growth of apple-rot fungi in liquid media.

The growth of some of the fungi was rather poor on the medium used, and in such cases the temperature response was small. It is interesting to note that *Sclerotinia cinerea*, *Glomerella cingulata*, and *Sphaeropsis malorum*, fungi which have given very decided temperature contrasts in other experiments, have in this case given practically none. *Neofabraea malicorticis* has also given little contrast but did not show great sensitiveness to temperature in the experiments on fruit. With the species of Botrytis, Penicillium, and Aspergillus used there was a very decided drop in growth between 25° and 30°. *Botrytis cinerea* and *Aspergillus niger* had an optimum at 25°, while the optimum for *Penicillium expansum* was 20° instead of 25° as in the experiments on fruit. None of the fungi made enough growth at 0° to produce a measurable quantity of mycelium. With many of them there was no evident growth. All of these, however, produced a vigorous growth when later removed to warmer temperatures.

PETRI-DISH EXPERIMENT.—It was found much more satisfactory to test the growth of the fungi on agar in petri dishes than by the use of flasks. Growth was more uniform, more easliy measured, and the results apparently were more reliable. In these experiments corn-meal agar was used for all of the fungi. The culture medium was poured into the dishes, and inoculations were made near the center of the plates as soon as the agar had cooled. Plates were made up in duplicates in all cases. They were allowed to stand in the laboratory for 24 hours after inoculation had been made and were then placed at the various temperatures indicated. In the first series of experiments no measurements of the colonies were taken at the end of this first 24 hours, but records were made after 4, 6, and 17 days. Figure 24 shows the results obtained at the end of 6 days after inoculation. In the second series of experiments measurements were taken at the end of the 24 hours at laboratory temperature and later measurements at the times indicated. The rate of development of the various fungi in this experiment is shown in figures 14 to 23. The graphs show the increase in the diameter of the colonies at the end of 2, 4, 6, 8, 18, and 34 days from the time of placing the cultures at the given temperatures. The comparative temperature responses of the different fungi is brought out in figure 25. In this figure the curves show the average daily increase in diameter after the end of the first day in storage. By not including the growth made in the day at laboratory temperature nor the growth in the first day at the storage temperatures it was thought that a more accurate record of the temperature responses of the fungi would be obtained. The base line represents temperature in all cases, and the perpendicular the diameter of the growth in millimeters.

A study of figures 14 to 23 as compared with figures 4 to 12 makes it evident that the rate of growth did not increase with time in the petri-dish experiments as it did in the experiments with apples, the fungi, with the exception of *Sphaeropsis malorum*, making as great increase in growth at both low and high temperatures during the first days of the experiment as they did during the later ones.

In comparing the growth on apples with that on corn-meal agar it will also be noted that in the latter case fewer of the fungi show a tendency to lower the minimum with the longer periods of time. This is partly accounted for by the fact that more of the fungi grew at the lower temperatures on the corn meal from the beginning of the experiment. *Fusarium radicicola* had made no evident growth on apples at 15° after five weeks nor *Glomerella cingulata* on apples at 10° after eight weeks, but both grew on corn-meal agar at 5° from the first. *Neofabraea malicorticis* and *Penicillium expansum* made no growth on apples at 10° after two weeks, but the former grew on corn-meal agar at 0° from the beginning of the experiment and the latter had made a start at that temperature by the end of the fourth day. *Volutella fructi* had made no

evident start on apples at 5° after two weeks, but grew on corn-meal agar at 0° from the first. *Sphaeropsis malorum*, however, showed the same slowness in starting on corn-meal agar at low temperatures that it had

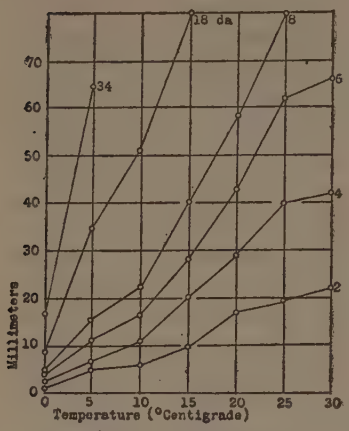

FIG. 14.—Graph showing the growth of *Alternaria* sp. on corn-meal agar in petri dishes.

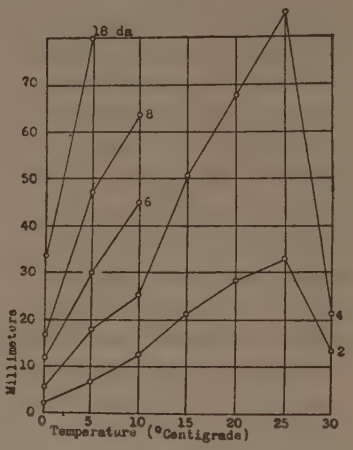

FIG. 15.—Graph showing the growth of *Botrytis cinerea* on corn-meal agar in petri dishes.

on apples and, contrary to the fungi already mentioned, it grew at a lower temperature on apples than on the agar. The above contrasts show that with nearly all of the fungi studied the period of incubation

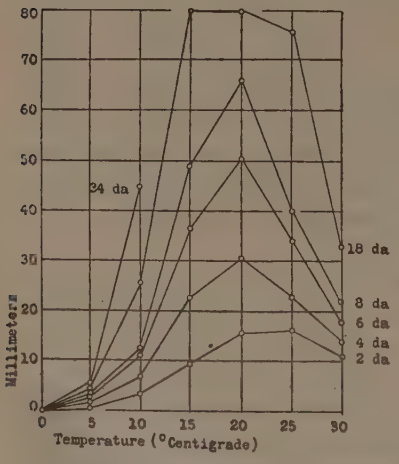

FIG. 16.—Graph showing the growth of *Cephalothecium roseum* on corn-meal agar in petri dishes.

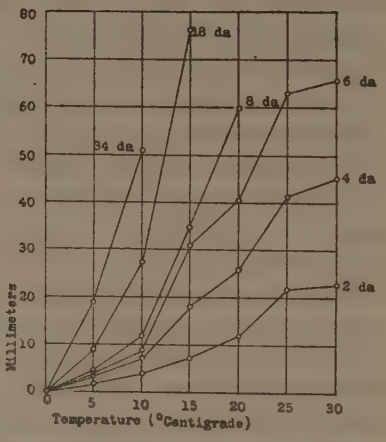

FIG. 17.—Graph showing the growth of *Fusarium radicicola* on corn-meal agar in petri dishes.

that was pointed out as important in the experiments with apples has much less significance when the same fungi are grown on corn-meal agar.

The contrast between the growth on apples and that on agar at low temperatures becomes still more striking when we consider that in the

plotting of the curves for figures 14 to 23 and also for figure 25 the growth during the 24 hours at laboratory temperature was always deducted from the final growth, a thing which could not be done in the case of the

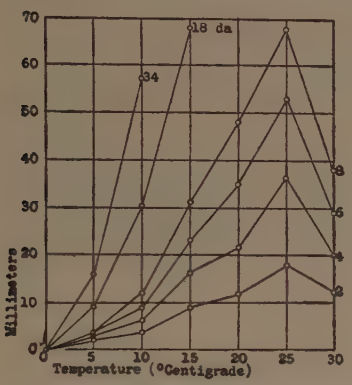

FIG. 18.—Graph showing the growth of *Glomerella cingulata* on corn-meal agar in petri dishes.

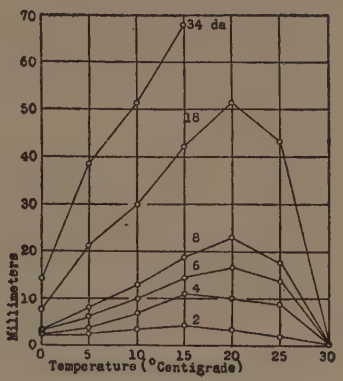

FIG. 19.—Graph showing the growth of *Neofabraea malicorticis* on corn-meal agar in petri dishes.

apple experiments, as the effects of the fungi were not evident at the end of one day.

It was thought that, since the agar probably remained practically uniform at the different temperatures and the apples were evidently

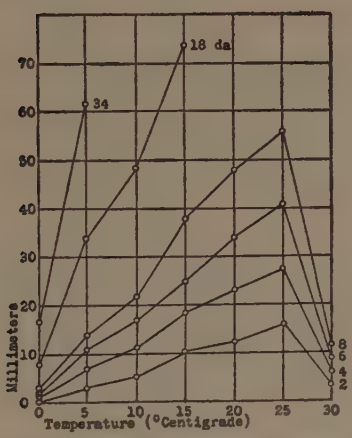

FIG. 20.—Graph showing the growth of *Penicillium expansum* on corn-meal agar in petri dishes.

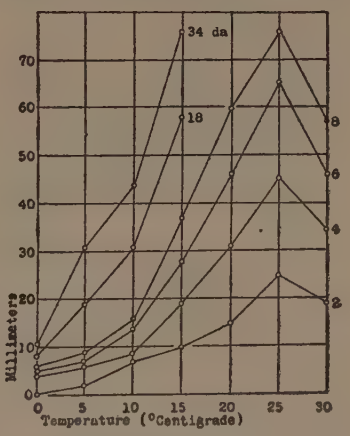

FIG. 21.—Graph showing the growth of *Sclerotinia cinerea* on corn-meal agar in petri dishes.

ripened and weakened by the higher ones, the optimum for some of the fungi might be lower on the former than on the latter and that there might be more sudden drops above the optimum with the former. A comparative study of the results, however, does not indicate this.

Some of the fungi that furnished little temperature contrast on the apples gave quite striking ones on agar. This is especially noticeable with *Cephalothecium roseum*, but it also occurs with *Alternaria* sp. and *Botrytis cinera*. On the other hand, the species of Sclerotinia, Penicillium, and Sphaeropsis used have given relatively less temperature contrast on corn-meal agar than on apples. (See figs. 1, 2, 25.) A medium favorable to the fungus apparently intensifies the temperature contrasts.

While there are the differences already pointed out in the temperature responses of the various fungi on the two media, the curves plotted for growth on corn-meal agar are on the whole in quite close agreement with those for growth on the fruit, showing that the temperature response is largely determined by the organism itself.

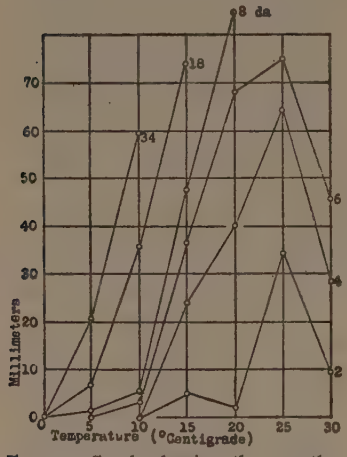

FIG. 22.—Graph showing the growth of *Sphaeropsis malorum* on corn-meal agar in petri dishes.

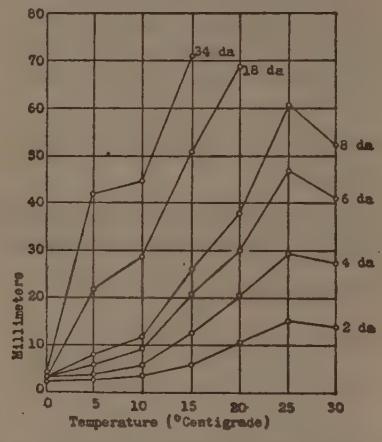

FIG. 23.—Graph showing the growth of *Volutella fructi* on corn-meal agar in petri dishes.

It will be noted that the curves for the petri-dish experiments, like those for the apple rots, have been plotted on the basis of the diameter rather than the area, thus giving relatively low values to the growth in the larger colonies and making the contrasts too small between the high and low temperatures and between the early and late stages of the experiment.

GERMINATION AT LOW TEMPERATURES

The above experiments with petri dishes furnish data as to the growth of the fungi at various temperatures when the cultures had been exposed for 24 hours in the laboratory before being placed at the temperatures of the experiment. A later test was made on corn-meal agar in which the petri dishes were inoculated as already described but were placed at the desired temperatures immediately. Notes were taken at the times indicated in Table IV.

TABLE IV.—*Results of germination experiments with various apple-rot fungi.*[a]

Fungus.	Germination at 10° C. after—		Germination at 5° C. after—		Germination at 0° C. after—	
	10 days.	31 days.	10 days.	31 days.	22 days.	31 days.
Alternaria sp	+	+	+	+	−	+
Aspergillus niger	−	−	−	−	−	−
Botrytis cinerea	−	+	−	+	−	+
Cephalothecium roseum	−	+	−	+	−	−
Fusarium radicicola	−	+	−	+	−	−
Glomerella cingulata	+	+	+	+	−	−
Penicillium expansum	+	+	+	+	+	+
Pestalozzia funerea	−	+	−	+	−	−
Sclerotinia cinerea (apple)	−	+	−	+	−	+

[a] + indicates germination; − indicates failure to germinate.

There was little contrast between the germination at 10° and that at 5°. At both these temperatures *Alternaria* sp., *Glomerella cingulata*, and *Penicillium expansum* had germinated by the end of 10 days and all of the fungi but *Aspergillus niger* by the end of 31 days. At 0° none of the fungi but *P. expansum* had germinated at the end of 22 days and only the species of Alternaria, Botrytis, and Sclerotinia were added to this by the end of 31 days.

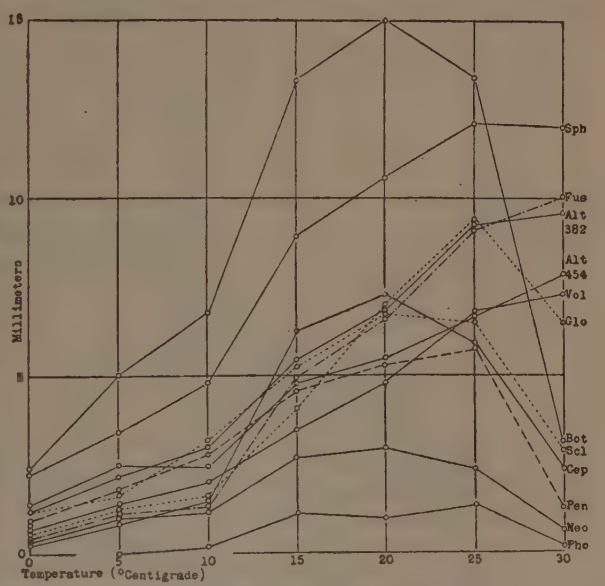

FIG. 24.—Graph showing the comparative growth of the different rot fungi on corn-meal agar petri dishes, first experiment. The curves show the average daily increase in diameter during the first six days of the experiment.

At 10° *Alternaria* sp., *Botrytis cinerea*, *Fusarium radicicola*, *Penicillium expansum*, and *Sclerotinia cinerea* were fruiting by the end of the month, and *Cephalothecium roseum* had made a good growth, but *Glomerella cingulata* and *Pestalozzia funerea* had produced colonies but a few millimeters in diameter. After 31 days at 5° the species of Alternaria, Botrytis, Penicillium, and Sclerotinia used were fruiting, while the colonies of Cephalothecium, Fusarium, Glomerella, and Pestalozzia had barely made a start. At 0° *P. expansum*

had produced colonies 3 mm. in diameter and was fruiting by the end of the month; *B. cinerea* and *S. cinerea* had produced colonies 0.5 mm. in diameter, but *Alternaria* sp. had little more than germinated.

A second series of germination tests was made in which the above results were confirmed. In this experiment it was also found that the

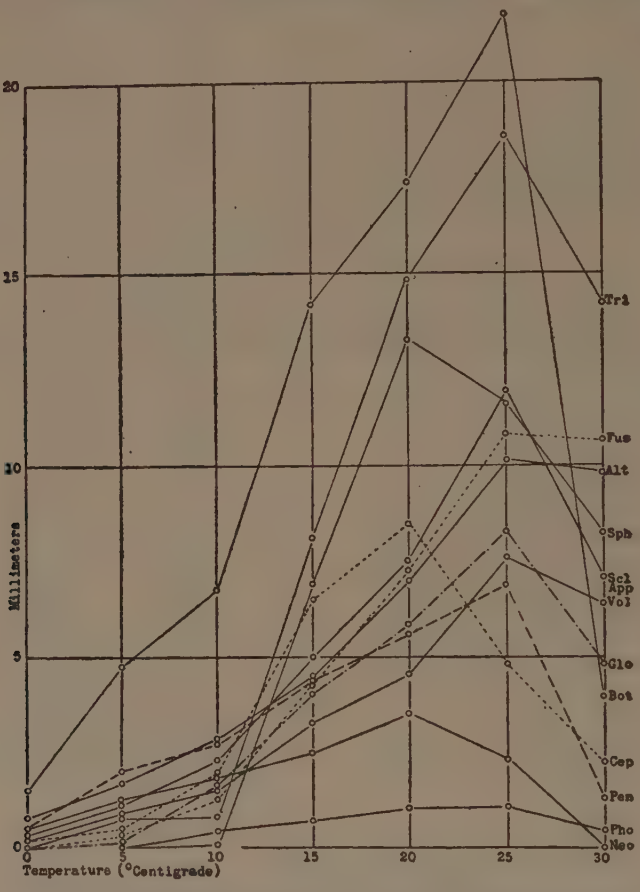

FIG. 25.—Graph showing the comparative growth of the different rot fungi on corn-meal agar petri dishes, second experiment. The curves show the average daily increase in diameter. With *Botrytis cinerea* and *Trichoderma* sp. they are based on the increase in diameter between the second and fifth days after inoculation, with *Sphaeropsis malorum* on the increase between the second and seventh days, and with all the other fungi on the increase between the second and eighth days.

spores of *Neofabraea malicorticis* germinated at 0° and that the fungus had fruited at that temperature at the end of 60 days.

The above results show that with a favorable medium *Penicillium expansum* is able to make a better start at 0° than any of the other fungi. The fact helps to explain its common occurrence in cold storage and is in agreement with the idea already suggested that its failure to grow on rather immature fruit at 0° is due in part to its weak parasitism.

DISCUSSION OF RESULTS

A comparison of the different tables and figures shows that the temperature response of a particular organism is modified by the medium upon which it is grown. The contrasts have been particularly striking between the behavior of the fungi on the living fruit and on dead organic matter. On corn-meal agar most of the fungi have grown from the first day even at rather low temperatures, while on apples there has usually been an initial incubation period of very slow growth which gradually passed over into a later one in which decay proceeded rapidly. In the initial stages of growth on the fruit the fungus must live almost entirely as a parasite and has not had time to build up reserve food material or secrete enzymes in quantity, while in the later stages it has a continually increasing mass of mycelium and the possibility of obtaining much of its food saprophytically from the tissue already broken down. The initial incubation stages of decay have been much more inhibited by low temperatures than the later stages, leading in some cases to a delayed development of rot and in others to a total prevention of it at a temperature at which the fungus was capable of making a rapid saprophytic growth. The inhibition at low temperatures was more pronounced with immature fruit than with mature specimens and with weak parasites than with strong ones.

Glomerella cingulata was unable to make any growth at 5° or 10° and *Fusarium radicicola* at 15° on Yellow Newtown or Winesap apples, but both fungi grew well on corn-meal agar at all three of these temperatures. *Neofabraea malicorticis* and *Penicillium expansum* had produced no evident rot on Winesap apples at 0°, 5°, or 10° at the end of two weeks, but they finally caused fruit decay at all these temperatures. Both grew from the first day at 5° and 10° when inoculated into corn-meal agar, and both had made a measurable growth on this medium at 0° by the end of the second day. Similar contrasts were found with several of the other fungi, but were not evident with *Sclerotinia cinerea* and *Sphaeropsis malorum*, two of the more distinctly parasitic fungi tested. The former was comparatively little delayed at low temperatures on either fruit or corn-meal agar; the latter was greatly delayed at 0°, 5°, and 10° on both food materials.

The early stages of the rots were not only partially inhibited by low temperatures but sometimes entirely prevented at temperatures at which the later stages could develop. In the commercial cold-storage experiments it was found that *Penicillium expansum* was able to make a good growth at 0° on fruit on which it was entirely unable to make a start at that temperature. This could not have been a question of germination, as the fruit was in the center of the barrel, and there would have been ample time for this to have taken place before the cooling was completed. This fact is very significant in showing the value of immediate as com-

pared with delayed storage. Most of the rot fungi are able to make but a very slow growth at 0° and 5° if placed at once at those temperatures; but if they go into storage with the accumulated energy of a week's growth or more the results are likely to be very different.

With the weakest parasites the maturity of the fruit has also had a modifying effect, the fungi having a lower minimum on the ripe than on the green fruit. This was evident with the apples of a particular variety inoculated at the same time and is also the most probable explanation for some of the contrasts in the results on the different varieties tested. At 0° *Penicillium expansum*, for instance, was able to produce a measurable rot on the York Imperial and Ben Davis by the end of 5 weeks, on the Yellow Newtown and Winesap that were slightly greener by the end of 8 weeks; but on still less mature York Imperial and Arkansas apples it had produced no rot at this temperature after 18 weeks.

Experiments have shown that practically all of the rot fungi are able to germinate at 5° and most of them at 0°. Germination is somewhat delayed at the latter temperature; but this does not seem to be the whole explanation for delayed growth on fruit at low temperatures, since in most of the experiments made time was given for germination before the fruit was placed in storage. It is of interest to note that while *P. expansum* was the first to germinate at 0° it is comparatively slow in making a start on fruit at that temperature. With *P. expansum* and probably with others of the weaker parasites cold storage inhibits the initial stages of the rotting more than it does germination.

While the minimum temperature for the various fungi has varied greatly with the food material, there has been little shifting of the optimum under the same conditions. *Alternaria* sp. and *Fusarium radicicola* have an optimum at 30° or above; *Botrytis cinerea*, *Glomerella cingulata*, *Penicillium expansum*, *Sclerotinia cinerea*, *Sphaeropsis malorum* and *Volutella fructi* at 25°, and *Cephalothecium roseum* and *Neofabraea malicorticis* at 20°.

The temperature curves are all plotted on the basis of diameter of the rot or of agar colony. On this basis the increased growth with a rise of temperature usually comes within the limits of the Van't Hoff law, the diameter becoming two to three times greater with each 10° rise in temperature; but as has already been pointed out, this method of estimation does not give full value to the increase in size in the larger rots and larger agar colonies. Curves plotted on the basis of area would come within the limits of the Van't Hoff law in most cases; but there would be some striking exceptions, *Sphaeropsis malorum* producing a rot area 16 times as great at 25° as at 15°, and *Botrytis cinerea* an area 25 times as great at 20° as at 10°.

SUMMARY AND CONCLUSION

(1) The behavior of *Alternaria* sp., *Botrytis cinerea*, *Cephalothecium roseum*, *Fusarium radicicola*, *Glomerella cingulata*, *Neofabraea malicorticis*, *Penicillium expansum*, *Sclerotinia cinerea*, *Sphaeropsis malorum*, and

Volutella fructi has been studied at different temperatures and under various conditions.

(2) In the inoculations on apples all of the fungi grew at 0° except the species of Fusarium and Glomerella, the former making no growth at 15° and the latter none at 10°. *Sphaeropsis malorum* had produced no evident rot at 15° by the end of one week nor the species of Penicillium and Neofabraea at 10° by the end of two weeks, while *Sclerotinia cinerea* produced measurable rots at 5° in one week and at 0° in two weeks. *Neofabraea malicorticis* had an optimum at 20°, *Fusarium radicola* at 30°, and all the other fungi at 25°. With most of the organisms the growth rate dropped off rapidly above 25°, but with the exception of *N. malicorticis* all made some growth at 30°.

In commercial cold-storage experiments on rather immature fruit *Penicillium expansum* continued to develop at 0° in the case of delayed storage, but was unable to grow at that temperature in the case of immediate storage.

(3) On corn-meal agar in petri dishes all the fungi used but the species of Cephalothecium, Fusarium, Glomerella, and Sphaeropsis grew at 0°, while these four made a fair growth at 5°. The optimum and maximum temperatures for the various fungi were the same as in the fruit-inoculation experiments.

(4) The spores of the species of Alternaria, Botrytis, Penicillium, and Sclerotinia had germinated in corn-meal agar at 0° at the end of one month; but those of the species of Aspergillus, Cephalothecium, Fusarium, Glomerella, and Pestalozzia had not. At 5° germination had taken place with all of them but *Aspergillus niger* at the end of the month.

(5) The temperature responses of the various fungi have been greatly modified by the food material upon which they were grown. *Fusarium radicicola* and *Glomerella cingulata* had a lower minimum temperature on corn-meal agar than on fruit and the early growth of the species of Alternaria, Botrytis, Neofabraea, and Penicillium used was much less inhibited on corn-meal agar at low temperatures than on the apples. In the case of fruit inoculations with *Penicillium expansum* and others of the weaker parasites the minimum has varied with the maturity of the fruit.

(6) With most of the fungi the initial incubation stages of growth on the fruit have been more inhibited by low temperatures than the later ones. With the weaker parasites like *Penicillium expansum* it is apparently this initial stage of decay rather than germination that determines the minimum temperature for fruit rot.

(7) The results show the importance of immediate as compared with delayed storage; the value of temperatures of 5° or 10° in short periods of storage and of 0° in longer ones and that the minimum temperature will vary with the prevalent fungus and with the variety and maturity of the fruit.

PLATE 1

A.—Grimes Golden apple affected with blackrot, showing character of the natural infections with *Sphaeropsis malorum* that were used in the temperature experiments.

B.—Ben Davis apple affected with bitter-rot, showing the character of the natural infections with *Glomerella cingulata* that were used in the temperature experiments.

A
B

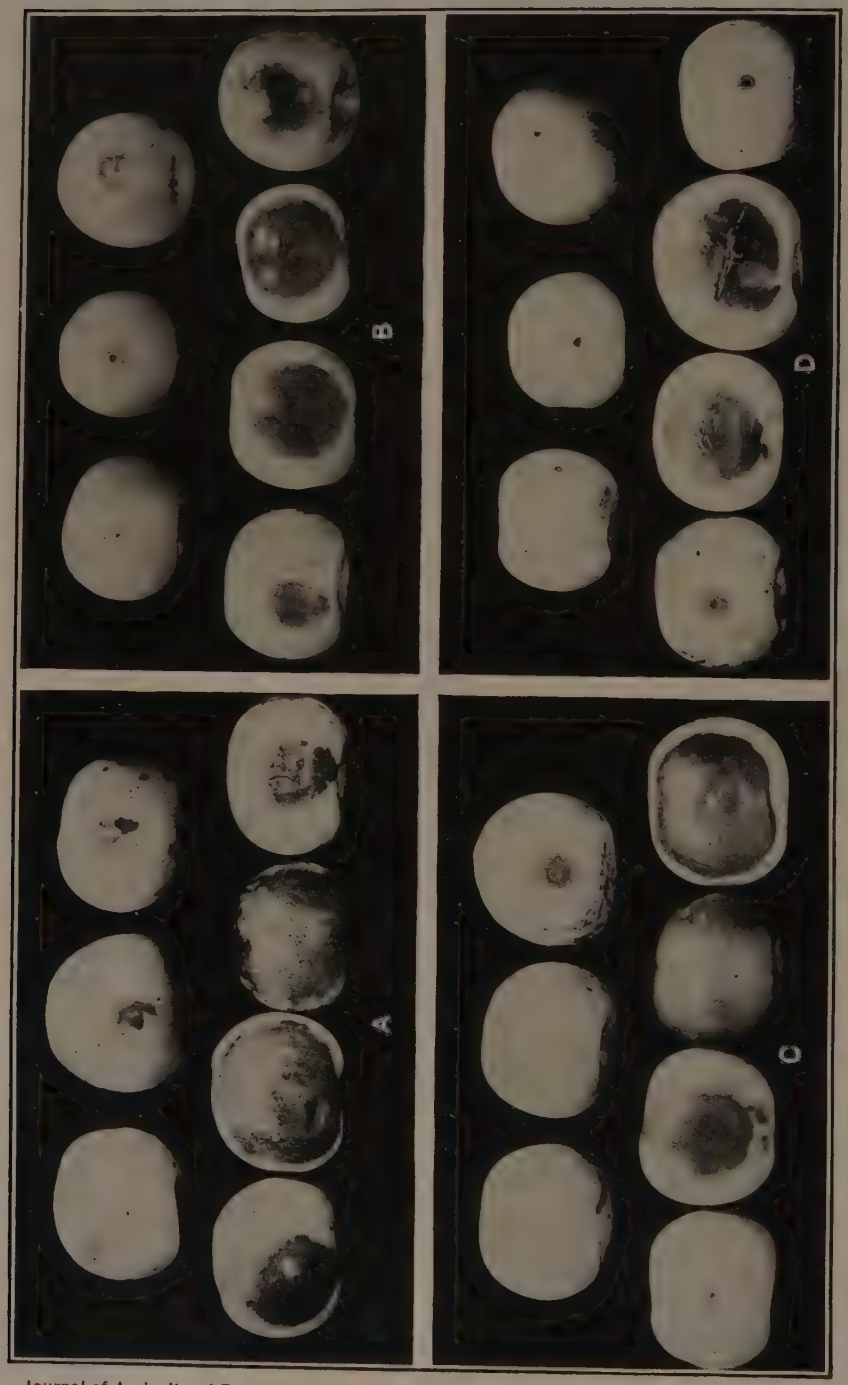
B
D
A
C

PLATE 2

Yellow Newtown apples, showing the behavior of *Sclerotinia cinerea* at different temperatures, the apples being placed in the order of the temperatures used:

A.—Results obtained with a culture of *S. cinerea* from a peach after two weeks at 0°, 5°, 10°, 15°, 20°, 25°, 30° C.

B.—Results obtained with a culture of *S. cinerea* from an apple after one week at 0°, 5°, 10°, 15°, 20°, 25°, 30° C.

C.—Results obtained with a culture of *Sphaeropsis malorum* after two weeks at 0°, 5°, 15°, 10°, 20°, 25°, 30° C.

D.—Results obtained with a culture of *Penicillium expansum* after two weeks at 0°, 5°, 10°, 15°, 20°, 25°, 30° C.

PLATE 3

Yellow Newtown apples, showing the behavior of apple-rot organisms at different temperatures, the apples being placed in the order of the temperatures used.

A.—Results obtained with a culture of *Volutella fructi* after two weeks at 0°, 5°, 10°, 15°, 20°, 250, 30° C.

B.—Results obtained with a culture of *Glomerella cingulata* after two weeks at 0°, 5°, 10°, 15°, 20°, 25°, 30°, C.

C.—Results obtained with a culture of *Neofabraea malicorticis* after three weeks at 0°, 5°, 10°, 15°, 20°, 25°, 30° C.

D.—Results obtained with a culture of *Neofabraea malicorticis* after five weeks at 0°, 5°, 10°, 15°, C.

B
D
A
C